

Transition-ready technologies and supporting capabilities at Sandia National Laboratories for HSARPA BAA 04-18

I. Partnering opportunities with Sandia National Laboratories

With long-term programs in chemical and biological national security, Sandia National Laboratories has developed a number of transition-ready technologies that could accelerate and enhance industry solutions to the challenges posed in the HSARPA Instantaneous Bio-Aerosol Detector Systems (IABDS) Broad Agency Announcement 04-18 (BAA 04-18) , including:

- Technologies related to μ ChemLab, a hand-held microchip-based instrument for detecting and identifying proteins and other biomolecules
- UV laser induced fluorescence technology for detecting biological agents
- Technologies to enhance fiber-based laser sources of infrared, visible, and ultraviolet radiation for detection and other applications
- Deep Ultraviolet (235 – 300 nm) Light Emitting Diode Capability
- Aerosol test chamber facilities used to create data for better solving problems surrounding force protection and facility response and restoration after a chem/bio weapon incident

Each of these technologies is described in greater detail below.

Collaborating with Sandia to integrate these technologies into national security solutions offers industry—and the nation as a whole—a number of benefits:

- **Faster, more economic development cycle:** Through decades of exploring national security issues, Sandia scientists have built up considerable expertise. Organizations that can tap this expertise will develop solutions more quickly and at less cost, ultimately accelerating the nation's ability to respond to national security challenges.
- **More robust solutions:** Sandia applies its renowned expertise in systems analysis and engineering to create practical, enduring solutions for the real world. By insisting that development efforts take into account the often less-than-ideal deployment context, Sandia can help industry develop more robust solutions.

- **Innovation through interdisciplinary teams:** Sandia scientists and engineers routinely work across disciplines to solve difficult challenges. Industry partners with reach into this profound blend of expertise can discover new opportunities for innovative solutions.
- **Long-term solutions:** As a national laboratory, Sandia is charged to perform the long-term research that is beyond the reach of most industrial entities. A partnership with Sandia on one project could lead to additional collaborations to create long-term solutions to today's problems and tomorrow's challenges.
- **Leveraged ROI:** Sandia technology development results from multi-year investments by federal agencies. Use of these technologies in solutions that bolster national security provides industry—and the taxpayer—an excellent return on investment.

This document will describe in detail the transition-ready technologies and supporting capabilities at Sandia that are relevant to this BAA. It then provides a brief overview of Sandia National Laboratories' comprehensive Chemical and Biological National Security program. The final section summarizes HSARPA guidelines and restrictions governing bidder partnerships with Sandia National Laboratories.

(For more information on Sandia's Chemical and Biological National Security program, please visit our website: www.ca.sandia.gov/chembio)

II. Transition-ready Technologies and Supporting Capabilities at Sandia National Laboratories

A. μChemLab™ Bio Detector System

Several key transition-ready technologies are related to the μChemLab™ bio detection system. Sandia National Laboratories is developing fully self-contained, portable, hand-held chemical analysis systems incorporating "*lab on a chip*" technologies. Our μChemLab systems utilize microfabricated substrates to provide sensitive devices with

fast response times in a low power, compact package. Currently, devices are being developed and tested for the detection of chemical and biological warfare agents, with the potential for analyzing chemical and biological compounds for multiple defense, environmental and medical applications.

Sandia researchers have miniaturized laboratory chemical analysis. The hand-held μ ChemLab Bio Detector spots molecules as dilute as 1 in 10 billion in less than 10 minutes and runs 8 hours on 4 watts of power supplied by lithium camera batteries. Multiple, microfabricated channels in the device separate molecules on the basis of charge/mass ratio and size using small-scale versions of a standard analytical approach, electrophoresis. A laser diode fluoresces the separated molecules as they emerge. On-board data processing identifies analytes of interest.

Key transition-ready technologies and supporting capabilities related to μ ChemLabTM include:

- The entire integrated system
- Underlying modules, including sample preparation systems, fittings, valves, pumps, mixers, concentrators, lysers, and optical subsystems
- Rapid “plug and play” design, assembly, and testing of novel concepts based on component architectures
- Design and engineering to produce prototypes of systems and underlying modules

The sections below offer greater detail on μ ChemLabTM and its components.

1. μ ChemLabTM System Design and Operation

Sandia has developed a hand-portable microchip-based analytical instrument, referred to as μ ChemLabTM, for the detection and identification of proteins and other biomolecules.

Proteins are labeled with a fluorescent dye (typically fluorescamine) and separated on a microfluidic-chip from other solution components according to molecular weight, using capillary gel electrophoresis (CGE), and mass/charge ratio using capillary zone electrophoresis (CZE). Following the separation, which typically occurs in 3 – 7 minutes, the labeled proteins are detected using laser-induced fluorescence (LIF), which provides fundamental (without preconcentration) picomolar range (10^{-11} M) detection sensitivity of fluorescent dyes and nanomolar sensitivity (10^{-9} M) for fluorescamine labeled proteins.

Proven Capabilities

To date, we have demonstrated the detection of biotoxins such as ricin, SEA, SEB, and tetanus; T-even phage bacterial viruses, as well as MS2, alpha *encephalitis*, and Vesicular Stomatitis, and *Vaccinia* viruses; and *B. anthracis* spores and vegetative cells.



Figure 1 shows the μ ChemLab™ hand-held modular bioagent detection device

Design

As shown in Figure 1, the μ ChemLab™ device has a modular design that provides reliability and flexibility, and facilitates rapid assembly, fluid and microchip replacement, troubleshooting, and sample analysis. Components include two independent separation modules that incorporate interchangeable fluid cartridges, a 2 cm square fused-silica microfluidic chip, and a miniature LIF detection module. A custom o-ring sealed manifold plate connects the chip access ports to a fluids cartridge and a syringe injection port, providing sample introduction and world-to-chip interface. Other novel microfluidic connectors include capillary needle fittings for fluidic connection between septum-sealed

fluid reservoirs and the manifold housing the chip, enabling rapid chip priming and fluids replacement. Programmable high-voltage power supplies provide bi-directional currents up to 100 μA at 5000 volts, enabling real-time current and voltage monitoring, and facilitating troubleshooting and methods development.

Analysis Methods

To enable rapid identification of bioagents with low false positive/negative rates, we have applied principal component analysis techniques that can, for example, distinguish between spores and vegetative cells. These methods are currently being augmented by algorithms based on Bayesian analysis methods, which can extract signatures from noisy data and thus improve the ability to separate signatures of interest in the presence of interferents. In addition, we developed a novel two-color labeling system, in which red-labeled standards are simultaneously detected in the presence of blue-labeled protein analytes. This allows inevitable variations in protein migration times to be corrected, dramatically reducing the potential for false positives/negatives.

2. $\mu\text{ChemLab}^{\text{TM}}$ subsystems

In addition to the analytical capabilities of $\mu\text{ChemLab}$, we are developing front-end components that allow automated sample processing including dye labeling, buffer adjustment, lysing, and preconcentration. The following component prototypes have been tested:

- Microfluidic solid-phase extraction cartridges for protein preconcentration
- Thermal- and chemical-based units capable of lysing viruses and spores
- Automated in-capillary protein labeler
- Size exclusion cartridge for separating proteins from small molecules.

An integrated prototype system including all of these components as well as pumps, automated valves, and computer control has been demonstrated using vegetative cells. A similar system for processing biotoxins and viruses is under development.

The system makes use of Sandia's liquid-phase $\mu\text{ChemLab}^{\text{TM}}$ as a general protein analysis tool. In this approach, $\mu\text{ChemLab}^{\text{TM}}$ is combined with an aerosol collector and an automated sample preparation system. Pathogens are collected from the air; constituent pathogen proteins are solubilized, labeled, and separated; and then the pathogens are identified from their characteristic protein signatures.

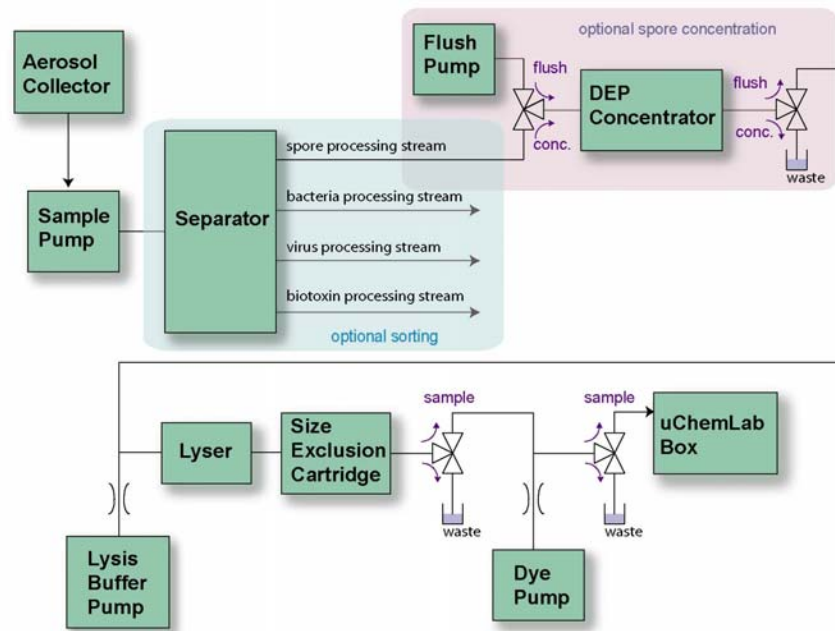


Figure 2 shows a schematic of the key elements of our $\mu\text{ChemLab}^{\text{TM}}$ approach

Figure 2 shows a schematic of the entire system. Spores, vegetative cells, and viruses can be processed together. Biotoxins may be processed separately. During the past year we have demonstrated use of these microfluidic sample preparation components both individually and on a system level in an integrated breadboard as shown in Figure 3.

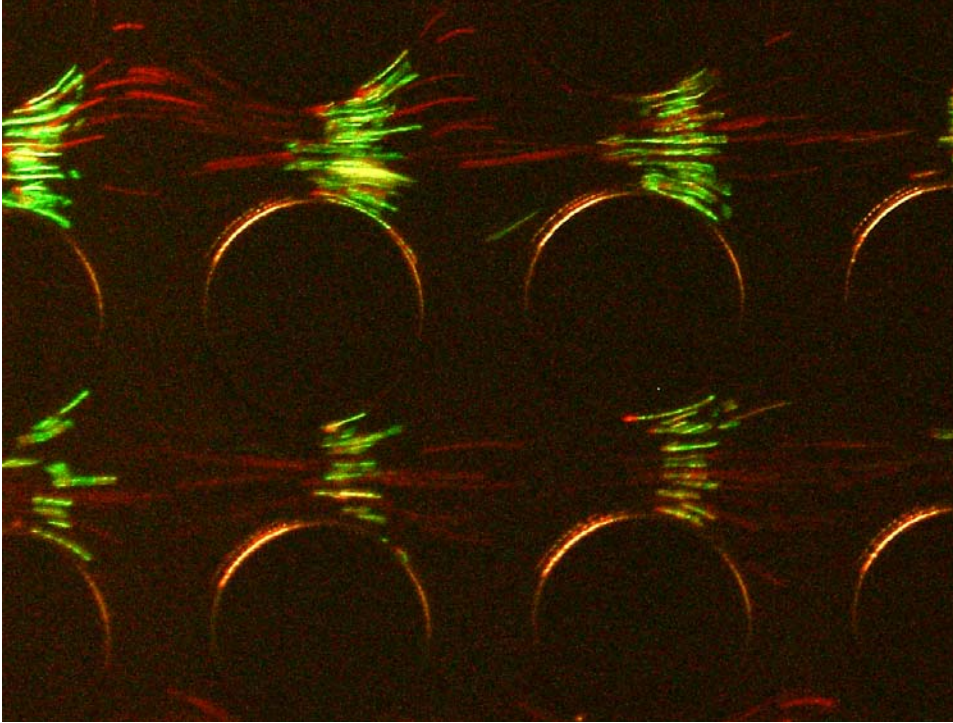


Figure 4 Selective dielectrophoretic trapping of Bacillus cereus (green) vs. Bacillus subtilis (red). Flow is from right to left at ~ 1 mm/s. The glass insulating circular posts are on 200- μ m centers.

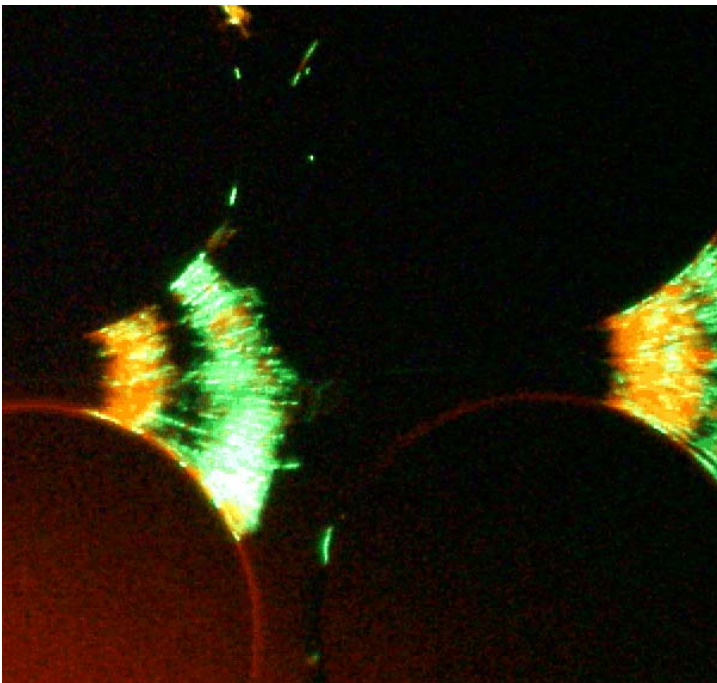


Figure 5 Dielectrophoretic sorting of live (green) and dead (orange) E. coli. Flow is from right to left at ~ 1 mm/s. The glass insulating posts are on 200- μ m centers.

Lysis - For this application, lysis involves more than just opening the cell; the constituent proteins must be solubilized. For vegetative bacteria and viruses, there are several options for solubilization. Spores, however, are much more difficult to solubilize. Spore coat proteins form a protective and highly cross-linked surface structure. Physical disruption methods such as heat or pressure can break open the structure so that DNA can be extracted, but physical disruption alone is not sufficient for recovering proteins. A chemical reducing agent is needed to break apart the disulfide linkages. A detergent is then used to solubilize the reduced proteins. In the past year, we have demonstrated effective lysis and solubilization of bacillus spores using either β -mercaptoethanol (BME) or Tris[2-carboxyethyl] phosphine (TCEP) as reducing agents. TCEP is preferable due to better compatibility with the subsequent labeling step. The process is done at near boiling temperature to speed the process. The lysis process has been demonstrated as both a bench-top procedure and in a micro-fluidic device. The device includes a temperature and pressure controlled lysis chamber of approximately 10 μ L volume and size exclusion chromatography (SEC) cartridges for subsequent cleanup of the reducing agent.

Size Exclusion Chromatography - Prior to injection into the μ ChemLab, the proteins are labeled with fluorescamine. The highly charged TCEP required for lysis of spores interferes with the subsequent labeling step. For this reason, we have developed miniature size exclusion chromatography (SEC) cartridges which remove TCEP in a flow through mode. In this application, SEC is not used to separate proteins for detection or identification; its only function is to remove TCEP in preparation for the subsequent labeling step. In SEC, the components of a mixture are separated according to their molecular size, based on the flow through a porous packing. Large molecules (proteins in this case) are excluded from interacting with the pores of the packing material, and therefore flow through the cartridge quickly. Small molecules (TCEP) enter the pores and therefore flow through the cartridge more slowly since their path through the column is longer.

Labeling - Labeling with fluorescamine dye is accomplished by simultaneously flowing the proteins and the dye stock solution (10 mM in dry acetonitrile) through a capillary

tube in proportions such that the final fluorescamine concentration is 1 mM. This capillary-based method is comparable to the benchtop labeling method.

Miscellaneous components - In addition to the specific components described previously, the sample preparation system has pumps, valves, power supplies, and controllers, all of which are shown in Figure 3. All of these microfluidic components have been developed and demonstrated.

(For more information on Sandia's μ ChemLab™ systems please go to:

www.ca.sandia.gov/chembio/tech_projects/detection/micro-chem-lab.html)

B. UV Laser Induced Fluorescence Capability

For many years, Sandia National Laboratories has been focused on developing UV laser induced fluorescence (UVLIF) technology to support the detection of biological agents. Recent efforts have included developing

- An extensive database and understanding of the fluorescence signatures of biological materials
- The detection technology necessary to field operational instrumentation

The technology Sandia has developed utilizes temporally-gated, spectrally resolved fluorescence measurements and elastic backscatter measurements. Classical least squares is used to fit the measured spectra to database signatures and multiple hypothesis testing and statistical tests are performed on the results of the fits to determine the best fit and to estimate confidence levels.

One application of Sandia's UVLIF technology is Ares, a van-portable bioaerosol detection system for outdoor monitoring at up to 5 km range. We have field tested the system in blind tests at the DoD-sponsored Joint Biological Standoff Detection System Production Qualification Test at Dugway Proving Ground. We are currently working with an industrial partner to commercialize the Ares system.

Sandia has also investigated the use of our UVLIF technology for short-range standoff or volumetric detection of biological aerosols in indoor or short-range outdoor applications. We have developed design concepts, modeled the performance by scaling results from our Ares system, and performed proof-of-principle experiments of eye-safe aerosol cloud detection using a 1.5 μm wavelength laser to detect small clouds and discriminate them from solid objects that may be present in an indoor environment.

*(For more information on Sandia's UVLIF technology please go to:
www.ca.sandia.gov/chembio/tech_projects/detection/Aura-Ares.html)*

C. Fiber-Based Laser Sources of Infrared, Visible, and Ultraviolet Radiation

Rare-earth-doped fiber amplifiers are widely used in applications requiring compact, rugged optical sources with high beam quality. However, use of these amplifiers in applications that require high-energy pulses (e.g., nonlinear frequency conversion) or narrow linewidth (e.g., spectroscopic sensing) has been limited by the low energy-storage capacity of single-mode fibers and by the onset of nonlinear processes in the fiber. Multimode (large core) fibers can mitigate these problems, but usually at the expense of beam quality.

To overcome these limitations, Sandia has developed a technique that uses bend loss (i.e., coiling of the fiber) as a form of distributed spatial filtering to suppress all but the lowest-order mode of a highly multimode fiber amplifier. This technique yields single-transverse-mode (diffraction-limited) beam quality without loss of efficiency. Using this method, we have obtained high peak-power (>300 kW) pulses and have demonstrated efficient frequency conversion into the visible, near-UV, and deep-UV spectral regions.

We have invented a new technique for pumping of high-power fiber sources. In this approach, the pump beam (typically from a laser diode) is launched by reflection from a micro-mirror embedded into the inner cladding (which surrounds the fiber core). Using

this method, we have constructed fiber amplifiers operating in the 1050 nm and 1550 nm regions, including polarization-maintaining amplifiers.

We are currently working on methods to further extend the power-generating capabilities and wavelength coverage of both cw and pulsed fiber sources. This work includes development of broadly tunable, Q-switched and mode-locked fiber lasers.

A summary of Sandia capabilities and accomplishments follows:

- Development of method for scaling the peak and average capability of cw and pulsed fiber sources by at least two orders of magnitude while maintaining diffraction-limited beam quality and high electrical-to-optical efficiency
- Demonstration of a peak power, pulse energy, and average power of 306 kW, 255 μ J, and 2.2 W, respectively, from a fiber amplifier seeded by a pulsed microchip laser (1064 nm wavelength, 0.8 ns pulse duration)
- Efficient generation of the 2nd, 3rd, 4th, and 5th harmonics (532 nm, 355 nm, 266 nm, and 213 nm, respectively) of a fiber amplifier seeded by a microchip laser
- Development of a side-pumping method for efficiently coupling the light from one or more pump sources (laser diodes, diode bars, or fiber-coupled sources) into a double-clad fiber
- Construction of packaged, turn-key fiber amplifiers based on our side-pumping method
- Development of a method for preparing the ends of the fiber for high-power operation and to minimize optical feedback
- Development of polarization-maintaining, Yb-doped, double-clad fiber

D. Deep Ultraviolet (235 – 300 nm) Light Emitting Diode Capability

Sandia has developed a state-of-the-art capability in solid-state deep ultraviolet light emitting diodes. These devices provide a compact, light-weight and robust alternative to traditional UV lamps for a range of applications including fluorescence-based biosensing, water purification, epoxy curing and non-line of sight communications. Through a multi-year DARPA-funded program, our team has demonstrated milliwatt level output powers in the 275-300 nm spectral range, and emission wavelengths as short as 237 nm. Sandia's expertise lies in the development of high quality wide bandgap AlGaIn semiconductor alloys, grown by metal-organic vapor phase epitaxy, and novel light emitting device structures. We are interested in working with partners to optimize and customize device performance and to implement these novel devices into specific application platforms. Areas of optimization include the development of custom wavelength sources in the 235-300 nm region, as well as improvements in device efficiency and lifetimes to meet system requirements. Engaging in development efforts toward demonstrating deep UV laser diodes is also of interest and may be relevant for applications that would benefit from a compact, solid-state deep ultraviolet laser source.

E. Aerosol Test Chamber Facilities

Sandia has developed an aerosol test chamber facility used extensively to generate valuable data that provide insight into many problems. Specifically, such data highlight knowledge gaps relevant to responding to a chemical or biological weapons (CBW) incident, including force protection, critical infrastructure protection, and facility restoration. The 8-ft wide by 16-ft long by 8-ft high chamber consists of two compartments, each an 8-foot cube separated by an intervening wall. Ducting through a filtered air mover permits rapid air changes for material removal and concentration adjustment and control.

The facility, originally developed under the DARPA Immune Building project, includes a knockdown spray system within the aerosol test chamber that employs a modified version of Sandia National Laboratories DF200 decontamination technology to experimentally investigate the rapid mitigation and decontamination of airborne CBW agent simulants. This facility has been used to determine the efficacy of various spray mitigation techniques for interior building CBW releases.

The following objectives have been or are currently being demonstrated under several projects:

- Optimization of the decontamination formulation for indoor spray applications
- Development of a knockdown spray application method to achieve the greatest effect on the mitigation and decontamination of CBW agents, especially those in aerosol form
- Development of a conceptual design of a building system, which can rapidly apply the decontamination formulation in the proper area, at the proper rates, and at the appropriate time
- Exploration of methods of outdoor CBW agent cloud knockdown and decontamination
- Benchmarking interaction modeling of CBW agent cloud physics and decontamination parameters
- Assessing effects of spray system variables, such as electrostatic spray nozzle charge, spray droplet size, decontamination formula, and spray duration

Additionally, a 1m³ chamber constructed of polypropylene is situated within the south compartment of the large aerosol test chamber. This smaller chamber is currently fitted with a fluidized bed aerosol generator used to provide stable concentrations of surrogate weaponized spores. Spore concentrations are verified via TSI Aerodynamic Particle Sizer (APS) measurements. This deposition chamber provides a means to generate known and controlled aerosol concentrations that provide uniform depositions of spores and other test aerosol particles onto test surfaces placed in the chamber. These test surfaces with known spore concentrations are used to test surface sample collection methods. Three different spore collection methods are currently being evaluated: use of

moistened swabs, moistened wipes and HEPA vacuum. These collection methods are being measured on concrete, stainless steel, indoor/outdoor carpet and painted drywall.

Additionally, SNL has performed characterization of several spray systems measuring drop size and delivery rates. A Malvern Spraytech and TSI Phase Doppler Particle Analyzer have been used to make in situ measurements of sprays.

An additional chamber is planned. This new chamber is expected to expand Sandia's aerosol test chamber capability. The technologies incorporated in the aerosol test chamber may be leveraged to investigate important areas of concern such as environmental sampling and the decontamination of biological toxins and toxic industrial chemicals.

(For more information on Sandia's aerosol test chamber facility please go to:

www.ca.sandia.gov/chembio/tech_projects/detection.html)

III. Sandia National Laboratories Chemical and Biological National Securities Program

Sandia National Laboratories is a multi-program engineering and science laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the U.S. Department of Energy (DOE). Sandia also works for the Department of Homeland Security (DHS), Department of Defense (DoD), and other federal agencies and partners with other federal groups, universities, and private industry. Our national security mission has grown from responding to the threats of the Cold War to countering a host of new threats—some nuclear, others involving chemical, biological or radiological weapons of mass destruction, and still others that are acts of terrorism.

Building on its systems integration strengths, Sandia National Laboratories has developed a comprehensive Chemical and Biological National Security Program. Coupling innovative science with sound engineering, technology, and systems analysis expertise, Sandia develops, demonstrates, and delivers technologies to detect, deter, defeat, or

mitigate the impact of chemical or biological attacks. The broad program, underway since 1996, employs our unique capabilities to analyze, create, and integrate defensive systems. Capabilities in chemical and biological defense span basic investigations in biology and detection; expertise in systems analysis, engineering and computing; and training facilities for public health and emergency responders. Sandians have applied their expertise to projects addressing sensing, restoration, facility hardening, training scenarios, and enhanced exchange of public health data.

(For more information on Sandia's Chem/Bio Program please go to:

www.ca.sandia.gov/chembio)

IV. Partnering Guidelines

The Sandia capabilities/technologies listed in this document are relevant to HSARPA Instantaneous Bio-Aerosol Detector Systems (IBADS) Broad Agency Announcement 04-18 (BAA 04-18) and are available to any bidder who is interested in partnering with Sandia National Laboratories under the following HSARPA guidelines/restrictions:

- DHS strategic partner laboratories may not propose directly to this solicitation or participate in any manner in the development of responses to this solicitation outside of the process defined by HSARPA.
- DHS strategic partner laboratories may collaborate with HSARPA bidders by providing explicitly identified transition-ready technologies subject to DoE and DHS approval. It is on the initiative of the providing laboratory to identify which technologies are transition-ready.
- DHS strategic partner laboratories may collaborate with HSARPA bidders by providing explicitly identified and unique supporting capabilities subject to DoE and DHS approval. It is on the initiative of the providing laboratory to identify which supporting capabilities are available to HSARPA bidders.
- HSARPA will neither encourage nor discourage bidders from incorporating DHS strategic partner laboratory technologies. This inclusion of these technologies is at the

sole discretion of bidders in their evaluation of best value and best technical response to the government under this solicitation.